

Attorney Docket No.: DC0258US.NP
Inventors: Supattapone and Deleault
Serial No.: 10/553,591
Filing Date: January 17, 2006
Page 3

Amendments to the Specification:

Please replace the paragraph beginning at line 25 of page 1 with the following rewritten paragraph:

--Various methods have been developed to enhance the amplification of PrP^{Sc} to increase the sensitivity of detecting PrP^{Sc}. Saborio, et al. ((2001) *Nature* 411:810-3) disclose the use of a protein misfolding cyclic amplification (PMCA) method wherein prion-infected tissue homogenates containing PrP^C are combined with normal brain homogenates in the presence of TRITON® X-100 and sodium dodecyl sulfate and subjected to repeated cycles of incubation and sonication to convert PrP^C in normal tissue to PrP^{Sc}. Lucassen, et al. ((2003) *Biochemistry* 42:4127-35) disclose a modified version of the PMCA method wherein the normal and prion-infected tissue homogenates are incubated under non-denaturing conditions for the conversion of PrP^C in normal tissue to PrP^{Sc}. Further, purified proteins and cell-lysate systems have been used to convert PrP^C to PrP^{Sc} (~~Koeiske~~, Caughey, et al. (2000) *Curr Issues Mol Biol* 2(3):95-101; Horiuchi and Caughey (1999) *Structure Fold Des.* 7:R231-R240; Saborio et al. (1999) *Biochem Biophys Res Commun* 258:470-475). Optimal non-denaturing, cell-free conditions (KCl, MgCl₂, citrate buffer and sarkosyl) for the conversion of PrP^C to PrP^{Sc} have also been disclosed (Horiuchi and Caughey (1999) *EMBO J.* 18:3193-3203). Cordeiro, et al. ((2001) *J. Biol. Chem.* 276:49400-9) teach that sequence-specific DNA binding to recombinant murine prion protein converts it from PrP^C to the

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Page 4

soluble PrP^{Sc} isoform similar to that found in the fibrillar state. Further, Nandi et al. ((2002) *Biochemistry* 41:11017-11024) teach that the interaction between PrP^C and anions (sulfate/phosphate) in polyionic ligands such as sulfated glycosaminoglycan and DNA, induce unfolding of the prion protein and conversion to PrP^{Sc}. DebBurman, et al. ((1997) *Proc. Natl. Acad. Sci. USA* 94(25):13938-43) demonstrate that GroEL and Hsp104 (heat shock protein 104), significantly, but distinctly affect conversion of PrP^C to PrP^{Sc}.--